Sunlight amelioration of *Prymnesium parvum* acute toxicity to fish

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Although a number of physical and chemical factors can influence ambient toxicity associated with *Prymnesium parvum* blooms in aquatic systems, the role of natural sunlight on the magnitude and duration of aquatic toxicity is less understood, particularly in inland waters. We performed two experiments exposing cell-free filtrates from *P. parvum* cultures to different magnitudes and durations of natural sunlight. During one study, fish acute toxicity from cell-free filtrates exposed to full and partial (50%) natural sunlight for 8 h was completely removed. However, acute toxicity of a dark treatment level in the field (26°C) did not differ from toxicity of a lab dark control (4°C), indicating that temperature did not influence the stability of acutely toxic *P. parvum* toxins in this study. In a second experiment, cell-free filtrates were exposed to full sunlight for different periods of time, resulting in a monotonic decrease in the magnitude of acute toxicity with an increased duration of sunlight exposure. Acute toxicity to fish was completely ameliorated after just 2 h of exposure to full sunlight, highlighting the potential importance of site-specific conditions on the duration of *P. parvum* impacts to aquatic life. Further studies are warranted to understand site-specific influences and direct and indirect photolysis mechanisms on the stability of *P. parvum* toxins in aquatic systems.

KEYWORDS: prymnesins; photolysis; site-specific; reservoir water quality; harmful algae blooms

INTRODUCTION

Blooms of the harmful algal species *Prymnesium parvum* are responsible for fish kills at various locations throughout the world (Granéli and Salomon, 2010). Since the 1980s fish kills associated with *P. parvum* blooms have increased in the inland waters of Texas and other parts of the USA (Southard *et al.*, 2010; Roelke *et al.*, 2010c); however, most previous studies have been conducted under marine or estuarine conditions (Brooks *et al.*, 2010). This species releases toxins to the water column that produce various toxicological responses (Brooks *et al.*, 2010). Prymnesins is a term generally used to describe the various toxins released by *P. parvum* (Manning and La Claire, 2010), although more recent studies have begun examining toxicity associated with fractions from *P. parvum* cultures that apparently do not contain prymnesins (Schug *et al.*, 2010). In fact, it appears that *P. parvum* produces other allelochemicals (Henrikson *et al.*, 2010) which differ greatly from structures reported for the prymnesins (prymnesin-1,

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prymnesin-2) by Igarashi *et al.* (Igarashi *et al.*, 1999). For example, the prymnesins are complex molecules with numerous polyhydroxy and polycyclic ether groups and have very high molecular weights (e.g. prymnesin 1 = 2263.7; Igarashi *et al.*, 1999). Because there are no methods to quantify all prymnesins at this time (Schug *et al.*, 2010), toxicological activity from *in vitro* hemolytic assays and *in vivo* bioassays with model aquatic organisms is routinely used as surrogate measures of bioavailable toxin(s) concentrations in water (Brooks *et al.*, 2010).

Under stressful environmental conditions (e.g. nutrient limitation; non-optimal temperature, salinity or light), *P. parvum* may become more toxic to aquatic life, which is apparently attributable to greater production and/or release of toxin(s) (Granéli and Johansson, 2003a, b; Uronen *et al.*, 2005; Baker *et al.*, 2007; Granéli and Salomon, 2010; Valenti *et al.*, 2010a). For example, nutrient-limited media with salinities of coastal waters resulted in higher *P. parvum* toxicity to ciliates and other phytoplankton species than nutrient replete cultures (Granéli and Johansson, 2003a, b). More recent studies by our group reported similar observations with greater *P. parvum* toxicity to fish mortality and cladoceran reproduction under nutrient-limited conditions with salinity representative of inland water conditions (Valenti *et al.*, 2010a). Additionally, the effects of temperature, salinity and light on the growth and toxicity of *P. parvum* have been evaluated with various model organisms (Larsen and Bryant, 1998; Baker *et al.*, 2007).

Interestingly, the influence of light on toxicity associated with *P. parvum* is less defined than other chemical and physical factors. When Larsen and Bryant (Larsen and Bryant, 1998) exposed cultures of different strains of *P. parvum* to light, they observed an increased growth rate with increasing light (saturation occurred at about 200 μmol photons/m^2/s), but the magnitude of toxicity to *Artemia* sp. was not affected. Similarly, Baker *et al.* (Baker *et al.*, 2007) observed that optimal growth was reached at 200 μmol photons/m^2/s and the magnitude of toxicity to fish did not respond to light intensity. Rahat and Jahn (Rahat and Jahn, 1965) found that *P. parvum* growth was possible in the dark when glycerol was added to the culture medium, and these cultures were more toxic than those grown with alternating dark and light periods, indicating that light may not be a requirement for toxin synthesis and production. They also concluded that any toxicity assays performed from cultures grown in alternating light–dark periods only reveal the net results of apparent production and "inactivation" of the toxin (Rahat and Jahn, 1965). Another study also demonstrated that constant illumination with a fluorescent lamp reduced toxicity and "inactivated" the toxins, whereas toxicity was only observed with light and dark periods (Reich and Parnas, 1962). Similar observations were reported by Fistarol *et al.* (2003).

Although light intensity may not strongly influence site-specific toxin production by *P. parvum* and associated ambient aquatic toxicity relative to other physical and chemical factors, the results of these previous studies suggest that light intensity may influence the stability and fate of toxins released by *P. parvum* in aquatic ecosystems. Whereas such studies manipulating the light environment of whole cultures were important for identifying factors that affect the physiological status and resulting toxicity of *P. parvum* cells, they did not allow for evaluation of which factors affect the environmental fate of toxins once released into the water column. In fact, it appears that only one published study has performed light experiments with cell-free preparations from *P. parvum* (Parnas *et al.*, 1962), using artificial light and one wavelength of ultraviolet light. Employing *Gambusia affinis* to evaluate the effect of different light treatments on toxicity to a fish model, "photoinactivation" of the toxin(s) was reported and such observations were not affected by the presence of cells, cell pigments, oxygen or glutathione (Parnas *et al.*, 1962). Thus, it appears probable that photolysis of the prymnesins occurred, which reduced the concentration of bioavailable toxins, reduced toxin(s) exposure to fish and thus reduced the magnitude of observed aquatic toxicity. An understanding, however, of natural sunlight influences on the stability of toxins produced by *P. parvum* and associated aquatic toxicity is lacking.

The purpose of this study was to investigate the possible photolytic effect of natural sunlight on *P. parvum* cell-free filtrates from cultures grown under conditions comparable with those when blooms occur in Texas inland water bodies. During two different experiments, filtrates were either exposed to sunlight of different intensities or to sunlight for different durations of time. Because quantification of prymnesins is not analytically feasible at this time (Schug *et al.*, 2010), responses of a fish model were used as a surrogate for bioavailable toxin(s) concentrations (Grover *et al.*, 2007; Roelke *et al.*, 2007, 2010a, b; Errera *et al.*, 2008; Brooks *et al.*, 2010; Valenti *et al.*, 2010a, b). It was important to isolate excreted toxins from *P. parvum*, because this eliminated confounding results due to physiological status of the organism or toxin production when cells remain in whole culture.

METHOD

Prymnesium parvum culture

Prymnesium parvum cells originally obtained from The University of Texas at Austin Culture Collection of

Algae (strain UTEX LB 2797) were cultured in an incubator (VWR Model 2015, West Chester, PA, USA) at 20 ± 1°C in 2.4 psu artificial sea water (ASW; Berges *et al.*, 2001) in f/8 media (Guillard, 1975) on a 12:12 light:dark cycle. The salinity employed in these cultures was selected to represent conditions in inland water bodies when *P. parvum* blooms result in ambient toxicity to aquatic life (Roelke *et al.*, 2010a, c). Nutrient-limited conditions (f/8) were selected for culture media, because nutrient limitation has previously been observed by our group to result in comparatively higher aquatic toxicity than f/2 media (Valenti *et al.*, 2010a). After cultures reached late stationary growth phase, cells were removed using glass fiber filters (GF/C, Whattmann, VWR, West Chester, PA, USA), and filtrates collected in foil-covered Erlenmeyer flasks. Each experiment was initiated with cell-free filtrates from a common culture, which reduced potential effects of different microbial densities on toxin(s) biotransformation in these non-axenic filtrates. Flasks were stored under refrigeration (4 ± 1°C) in the dark for less than 24 h before the two sunlight studies were initiated.

Sunlight Experiment 1

Three light treatment levels were selected, including full sunlight, partial sunlight and no sunlight. The partial sunlight treatment was made by placing a shade cloth on a frame over the top of the beakers, which were selected as experimental units for incubation of cell-free filtrate from a common culture (initial *P. parvum* culture density = 1.9×10^5 cells/mL). The dark treatment level was held under a box beside the two other light treatments. To perform the sunlight exposures, 600-mL beakers filled with 500 mL of cell-free filtrate or ASW controls were placed in their respective light treatment level. The first sunlight exposure study was conducted on 29 April 2009 at the Baylor Experimental Aquatic Research (BEAR) facility, Waco, Texas, USA, for 8 h beginning approximately 1 h after sunrise. Light intensity was quantified throughout the study period with Hobo U30-GSM Cellular Remote Monitoring System (Onset, Bourne, MA, USA). At the end of the 8 h sunlight exposure period, beakers were covered with Parafilm, placed in the dark in an ice-filled cooler, and returned to the lab where they were stored overnight under refrigeration (4 ± 1°C) in the dark. Less than 24 h later, toxicity assays with the model test organism, the fat-head minnow *Pimephales promelas* were initiated with samples from the light exposure study. Previous studies indicated that holding toxic cell-free filtrates under refrigeration (4 ± 1°C) in the dark for several days did not reduce hemolytic activity (Hagström and Granéli, 2005) or for 28 d did not influence the magnitude of acute aquatic toxicity to *P. promelas* (Urena-Boeck, 2008). In addition, toxicity was assessed for filtrate that was stored at 4 ± 1°C in the dark after harvesting the cells, which hereafter is called "lab dark control".

Sunlight Experiment 2

Similar to Experiment 1, Experiment 2 was also conducted at the BEAR over an 8 h period on 27 August 2009, and other experimental details were consistent with those described for the first study (initial *P. parvum* culture density = 10.6×10^5 cells/mL). Only full sunlight and no sunlight treatment levels were examined and an experimental unit was removed from sunlight following either 0.5, 1, 2, 4 and 8 h of exposure to natural sunlight. At that time, beakers were covered with Parafilm, placed in the dark in an ice-filled cooler and returned to the lab where they were stored overnight under refrigeration (4 ± 1°C) in the dark. Less than 24 h later, toxicity assays with juvenile *P. promelas* were initiated with samples from the light exposure study, and for lab dark controls, which again was consistent with Experiment 1.

Laboratory toxicity bioassays

Acute toxicity of the filtrates was assessed with a modification of US EPA method 2000.0 (US EPA, 2002) using *P. promelas* as a model test organism. Less than 48-h-old juveniles (all hatched within 24 h) were allowed to feed on *Artemia* nauplii at least 2 h before initiation of the test at 25°C. The *P. parvum* cell-free filtrate for each treatment was diluted with ASW to dilutions of 100, 50, 25, 12.5, 6.25 and 3.13% filtrate. All solutions were adjusted to pH of 8.5, including the ASW diluent, before mixing the dilutions and filling the test chambers, because this pH has been demonstrated to result in greater toxicity to fish than lower pH levels (Valenti *et al.*, 2010a). Test chambers consisted of three replicate 100-mL beakers filled with 80 mL of test solution at each concentration for each of the four treatments. Beakers of light-exposed ASW and reconstituted hard water (RHW; APHA *et al.*, 1995) not exposed to ambient sunlight served as controls. Five organisms were placed in each experimental unit, and bioassays were performed in an incubator (Norlake®; Hudson, WI, USA) at 25 ± 1°C with a 16:8 L:D cycle for 48 h. Modification to the EPA test method was made by using a time-to-death study design in which mortality was assessed at several time points, rather than only at

24 and 48 h. Time points were 1, 2, 3, 4, 5, 6, 9, 12, 18, 21, 24 and 48 h. Fish mortality was recorded at every time point, but dead organisms were not removed until 24 h according to standard methods (US EPA, 2002). Toxicity assays performed on samples from the second light experiment closely followed those conditions of the first light study; however, bioassays for the second light study were performed in the dark.

Statistical analyses

Median lethal concentration (LC_{50}) values were calculated for all filtrates using an appropriate method of analysis based on attributes of the data (e.g. Spearmann–Karber or Probit; US EPA, 2002). Median lethal time-to-death (LT_{50}) values were estimated using the Trimmed Spearmann–Karber method (US EPA, 2002). Regression analyses were performed in SigmaPlot (Version 11).

RESULTS

Sunlight Experiment 1

During the sunlight study period, the mean ambient light intensity (±SD) for the full natural sunlight treatment level was 300 (±179) μE (overcast conditions). The mean partial sunlight treatment level was determined to be 51.5% of the mean full sunlight during the 8 h study period. Cell-free filtrates of *P. parvum* exposed to either full sunlight or ~50% sunlight did not result in significant *P. promelas* mortality during the 48-h acute study when ambient temperature did not exceed 26°C. In fact, there was not a single mortality observed in any bioassay of these light treatments (Fig. 1); all ASW and RHW controls also had 100% survival. However, the cell-free filtrate maintained in the dark adjacent to the natural sunlight treatment levels was highly toxic to fish with a 24 and a 48-h LC_{50} value of 19.50% filtrate [95% confidence intervals (CI): 14.99–25.38%]. Such a magnitude of toxicity to fish was similar to that of the cell-free filtrate stored in the dark under refrigeration at 4°C (dark lab control) in the laboratory during the natural sunlight study period: an acute 24 and 48-h LC_{50} value of 14.03% (10.29–19.13%) filtrate was determined. Results from the time-to-death study showed that the 100% treatment levels of both laboratory and field dark treatments all organisms were dead within 3 h. In the 25% filtrate treatment level for both dark treatments, 50% mortality (LT_{50}) was calculated at 2.3 h (95% CI: 1.37–3.84 h; Fig. 2).

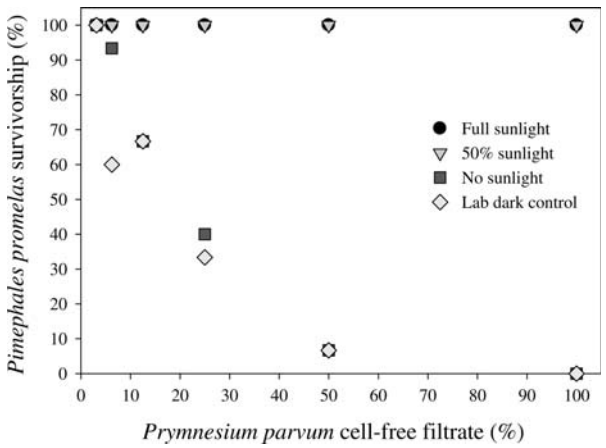

Fig. 1. *Pimephales promelas* 24 h survivorship following exposure to *Prymnesium parvum* cell-free filtrates previously treated with different natural sunlight intensities (up to 26°C) and a laboratory dark control (maintained at 4°C).

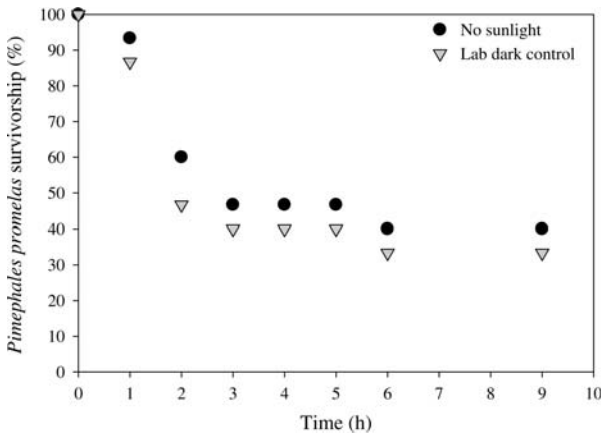

Fig. 2. *Pimephales promelas* survivorship for the first 10 h of the 48 h acute study when exposed to 25% filtrate of the no sunlight treatment level (up to 26°C) and a laboratory dark control (maintained at 4°C).

Sunlight Experiment 2

During sunlight study 2, mean ambient photosynthetically active radiation for the 0.5, 1, 2, 4, and 8 h exposure periods was 1472, 1762, 1805, 1752 and 1510 μE (full sun with no cloud cover), respectively. Similar to study 1, cell-free filtrates of *P. parvum* cultures were highly toxic to *P. promelas*, with a 24 and a 48-h LC_{50} value of 1.34 (% filtrate). With an increasing duration of exposure to natural sunlight, 24-h LC_{50} values of this cell-free filtrate increased until no mortality was observed at 2 h (Fig. 3). Similarly, times to 50% mortality (LT_{50}) in the 100% filtrates also increased with the duration of sunlight exposure (Fig. 3). Because both LC_{50} values and LT_{50} values were increased by sunlight treatment levels, *P. parvum* toxins likely were increasingly photolyzed with an increasing duration of sunlight

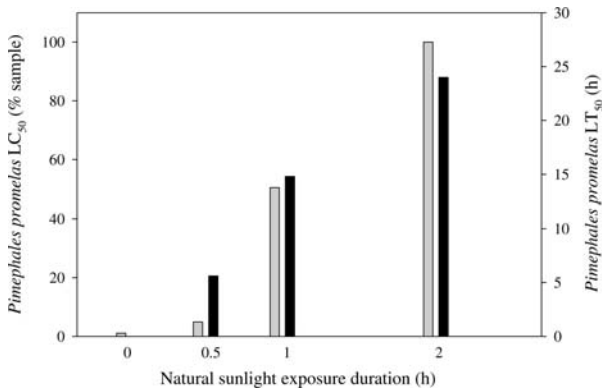

Fig. 3. *Pimephales promelas* 24 h LC_{50} values (grey bars) and LT_{50} values (black bars) for 100% cell-free filtrate of *P. parvum* cultures exposed to natural sunlight for 0.5, 1 or 2 h. Toxicity was not observed in 2 h treatment ($LC_{50} > 100\%$, $LT_{50} > 24$ h).

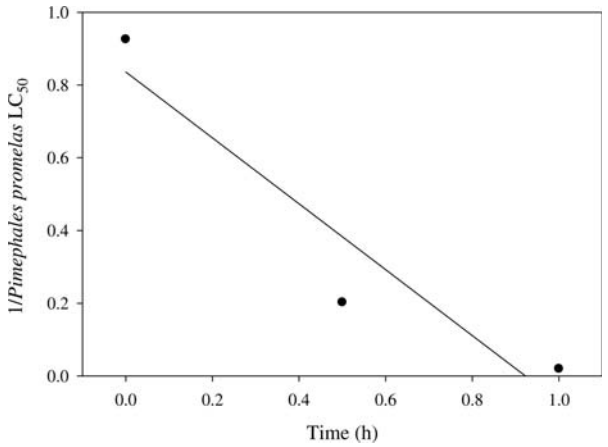

Fig. 4. 1/*Pimephales promelas* 24 h LC_{50} values for cell free filtrate of *P. parvum* cultures exposed to natural sunlight for 0, 0.5 or 1 h ($y = -0.9061x + 0.8359$; $R^2 = 0.8941$).

exposure. To compare the relationship between time and LC_{50} values to expectations for aquatic contaminants (Fig. 4), Fig. 4 depicts the inverse of 24-h *P. promelas* LC_{50} values, a potential surrogate for bioavailable toxin(s) concentrations, versus time ($y = -0.9061x + 0.8359$, $R^2 = 0.8941$).

DISCUSSION

Factors associated with *P. parvum* bloom dynamics have received an increased attention in inland waters over the past few years (Baker *et al.*, 2007, 2009; Roelke *et al.*, 2007, 2010a, b, c; Schwierzke *et al.*, 2010a; Schwierzke-Wade *et al.*, 2010b; Southard *et al.*, 2010; Valenti *et al.*, 2010a, b). Although the role of natural sunlight in ambient aquatic toxicity in inland waters associated with *P. parvum* has not previously been explored, an advanced understanding of site-specific factors, including sunlight, influencing bloom formation is needed to support predictive modeling and environmental management efforts (Grover *et al.*, 2010a, b). This consideration is critical because *P. parvum* is present in a number of inland water bodies (e.g. Lakes Waco and Somerville) and coastal areas of Texas (e.g. Galveston Bay) where blooms do not form (Roelke *et al.*, 2010b; Southard *et al.*, 2010). In the first experiment of this study, *P. parvum* cell-free filtrates maintained in the dark at 4 and 26°C were acutely toxic to fish whereas filtrates exposed to full or partial sunlight for 8 h did not result in fish mortality (Fig. 1). In the second experiment, toxicity to fish was completely ameliorated after just 2 h of exposure to full sunlight (Fig. 3).

The findings from both experiments in the present study are generally consistent with those of Parnas *et al.* (Parnas *et al.*, 1962). When a *P. parvum* supernatant was exposed to a tungsten lamp for just 1.5 h, toxicity to *Gambussia* sp. was removed, and such toxicity elimination was not influenced by the addition of cell pigments, oxygen or glutathione. Parnas *et al.* (Parnas *et al.*, 1962) further concluded that a photochemical process was involved in "inactivation" of toxins present in the *P. parvum* cell-free filtrate. However, Parnas *et al.* (Parnas *et al.*, 1962) could not suggest how photons may interact with the toxins because prymnesin structures were not reported until over 30 years later (Igarashi *et al.*, 1998). Our findings also suggest a mechanism explaining the immediate toxicity removal during a recent *P. parvum* bloom in Lake Whitney, TX, USA (Schwierzke-Wade *et al.*, 2010b). In the study by Schwierzke-Wade *et al.* (Schwierzke-Wade *et al.*, 2010b), nutrient loading associated with an inflow event appeared to stop toxin production by *P. parvum*, as observed in many other studies focused on the Texas strain (Grover *et al.*, 2007; Roelke *et al.* 2007; Errera *et al.*, 2008, Barkoh *et al.*, 2010; Kurten *et al.*, 2010). Specifically, the inflow event led to a 30% dilution of in-lake populations where post-inflow cell densities were still above the defined bloom levels (Schwierzke-Wade *et al.*, 2010b). Interestingly, the toxicity of pre-inflow water, however, was not reduced by 30%, but instead went from highly toxic to non-toxic, suggesting that the existing *P. parvum* toxins in the water column were rapidly photodegraded (Schwierzke-Wade *et al.*, 2010b), when the findings of the present study are considered. In the present study, the complete disappearance of acute toxicity to fish from cell-free filtrates exposed to just a few hours of full sunlight and the monotonic decrease in the magnitude of toxicity with an increased duration of sunlight exposure (Fig. 3) provides reasonable evidence that photodegradation, and potentially photolysis, of acutely toxic *P. parvum* toxins

occurred. Thus, sunlight appears to be an important factor associated with the magnitude and duration of *P. parvum* impacts to aquatic life.

Specific chemical groupings and classes, such as compounds with conjugated double bonds and aromatic rings, are more susceptible to photodegradation because their internal energy states correspond to the incoming light energy (Mill, 2000; Newman and Unger, 2003). Whereas direct photolysis occurs when photons directly interact with and break molecular bonds, indirect photolysis becomes important for chemicals when photons are absorbed by dissolved organic matter (DOM), such as humic acids, which then forms a reactive species, such as a hydroxyl radical, that facilitates the degradation of the contaminant (Mill, 2000; Newman and Unger, 2003). Direct and indirect photolysis sunlight rate constants, k_{PE} and k_d, respectively, and half-life ($t_{1/2}$) for surface water bodies are used to understand photolytic degradation of organic chemicals. Photolysis half-life is defined as:

$$t_{1/2} = \frac{0.693}{k_{PE}} \quad \text{or} \quad \frac{0.693}{k_d}. \qquad (1)$$

As noted above, the prymnesins described by Igarashi *et al.* (Igarashi *et al.*, 1999; prymnesin-1, prymnesin-2) have received most attention, although *P. parvum* appears to produce other allelochemicals (Schug *et al.*, 2010; Manning and La Claire, 2010; Henrikson *et al.*, 2010). Further, it is likely that additional allelochemicals will be attributed to *P. parvum* in the future (Valenti *et al.*, 2010b). Unfortunately, the lack of available prymnesins coupled with an absence of reliable analytical methods for quantification (Schug *et al.*, 2010) has precluded formal photolysis studies of prymnesins. It is not possible at this time to specifically identify photodegradation products of prymnesins described by Igarashi *et al.* (Igarashi *et al.*, 1999), particularly because they are such large molecules with many bonds that could be subject to breakage by photons. As one example, susceptible chromophores (or, a region that can absorb ultraviolet and visible light; Connell, 2005) are the series of single- and double-bonded carbons that is found on both ends of the reported structures of the prymnesins reported by Igarashi *et al.* (Igarashi *et al.*, 1999). On the basis of an understanding of photoreactions with other chemicals in surface waters, it appears reasonable that these regions absorb photons and probably cause degradation of the parent toxins (Mill, 2000), particularly if the more hydrophobic portion of prymnesins, containing a primary amine, is responsible for interactions with gill membranes and subsequent toxicity (Valenti *et al.*, 2010a). However, information in the present study provides an initial estimate of the influence of natural sunlight on degradation of acutely toxic *P. parvum* toxins.

As the duration of aquatic exposure to a chemical increases, the magnitude of aquatic ecotoxicological responses should correspondingly increase due to ecotoxicokinetic and ecotoxicodynamic principles (Rand, 1995). In both experiments of the present study, the onset of acute toxicity to fish was rapid, with complete mortality in the 100% filtrate within 3 h and 50% death in the 25% filtrates at ~2.3 h in the first study (Fig. 2). In the first experiment, sunlight appeared to reduce toxin concentrations to very low levels because fish mortality was eliminated by full and ~50% sunlight exposure. In addition, LC_{50} values were very similar in the dark treatment in the field ($LC_{50} = 19.5\%$, 95% CI = 10.29–19.13%) and the dark laboratory refrigerated control ($LC_{50} = 14.03\%$; 95% CI = 14.99–25.38%), highlighted by overlapping 95% CIs. Such comparable toxicity to fish suggests that temperature, which was maintained at 4°C in the lab and ~26°C in the field, did not influence the stability of acutely toxic *P. parvum* toxins in this study. Toxins produced by *P. parvum* are known to result in a rapid onset of toxic effects because the toxins are suggested to exert toxicity at the level of the gill, which is a vulnerable surface directly exposed to bioavailable chemicals in aquatic systems (Terao *et al.*, 1996).

If an aqueous concentration of a chemical remains stable, aquatic toxicology theory would anticipate an increasing relationship between $1/LC_{50}$ and duration of exposure (Rand, 1995). No appreciable acute toxicity was observed after 3 h during an initial time-to-death study with fish, which is contrary to expectations (Rand, 1995), and suggests that *P. parvum* toxins were influenced by incubator light conditions (Fig. 2). Subsequently, bioassays of samples from the second sunlight experiment, which manipulated the duration to which samples were exposed to full sunlight, were performed in the dark. Contrary to the expected increasing relationship between $1/LC_{50}$ and duration of exposure (Rand, 1995), the observed relationship between $1/LC_{50}$ versus time is *opposite* to that expected for a stable chemical (Fig. 4). Because the magnitude of toxicity may serve as a useful surrogate for the concentration of bioavailable toxins in a sample, the magnitude of toxic response may provide a biosensor for toxin concentrations (Baker *et al.*, 2007; Roelke *et al.*, 2007; Brooks *et al.*, 2010; Valenti *et al.*, 2010a, b). Consequently, the relationship depicted in Fig. 4 provides for initial estimation of an aggregate photolysis half-life ($t_{1/2}$) for *P. parvum* toxins. On the basis of the conditions of natural sunlight exposure in this study (mean PAR = 1762 μE), an aggregate and conservative $t_{1/2}$ of 0.37 h is proposed for *P. parvum* toxins, exposed to full sunlight. Thus, the photodegradation $t_{1/2}$ of *P. parvum* toxins in an

inland water body would be expected to be longer than this time period and to vary among the various prymnesins with site-specific light penetration with depth.

It is not possible to determine whether direct or indirect photolysis was primarily responsible for the photodegradation of *P. parvum* toxins observed here. Moreover, indirect photolysis would vary with the composition of DOM and thus formation of reactive oxygen species (ROS; e.g. oxyl- and peroxyl radicals, superoxide radical anions, hydroxyl radicals) in differing natural habitats. The findings in the present study with cell-free filtrates of *P. parvum* contrast with those observations where light exposure increased the hemolytic activity of the Raphidophyte *Heterosigma akashiwo*, although a ruptured cell suspension was tested by Ling and Trick (Ling and Trick, 2010). Such observations may be associated with an increased toxicity following sunlight generation of ROS (Ling and Trick, 2010). Ultimately, the availability of prymnesin standards for various types of molecules produced by *P. parvum* would facilitate the investigation of stability and photodegradation in natural waters.

The observed loss in toxicity to fish in this study when exposed to sunlight suggests that nighttime toxin release of toxins by *P. parvum* cells may be relevant to the environmental management of inland waters experiencing fish kills. Producing large-molecular-weight compounds such as the prymnesins described by Igarashi *et al.* (Igarashi *et al.*, 1999) would be energetically costly, so it would be a more advantageous life history strategy to release the toxins when sunlight exposure would be minimal; however, the relationship between diurnal toxin synthesis and release by *P. parvum* is not understood. This question deserves additional study because an advanced understanding of the factors influencing toxin production and photodegradation rates, and thus the magnitude and duration of toxin concentrations, ultimately resulting in ambient aquatic toxicity, is needed to provide important information for environmental management of fish kills and other impacts on aquatic life resulting from *P. parvum* blooms.

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